In Vitro Antimicrobial Activity of Zodia (*Evodia suaveolens*) Leaf Extract on Pathogenic Agents Dragon Fruit Plant

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ABSTRACT

The use of antimicrobials from plant extracts has not been used optimally to control pathogenic agents in dragon fruit plants. The purpose of this research was to determine the antimicrobial activity of zodia (Evodia suaveolens) leaf extracts on pathogenic agents of dragon fruit plants in vitro. The research method is laboratory research with Completely Randomized Design (CRD). The antimicrobial concentrations of zodia (E. suaveolens) leaf extract used six types including 50%, 60%, 70%, 80%, 90%, and 100% with four replications. The research sample was the leaf of zodia (E. suaveolens), Pseudomonas aeruginosa and Fusarium oxysporum strain Malang. Test the antimicrobial activity of zodia (E. suaveolens) leaf extracts on the growth of P. aeruginosa and F. oxysporum using the disc-diffusion method and wells method. The research instrument was used the observation sheet of the diameter of inhibition zone indicated by the clear zone. Diameter of inhibition zone data were analyzed using the One Way ANOVA test. The results showed that the antimicrobial activity of zodia (E. suaveolens) leaf extract significantly inhibited the growth of P. aeruginosa and F. oxysporum (P <0.05). These results recommend zodia (E. suaveolens) leaf extract as an antimicrobial agent for dragon fruit plant pathogens.

Introduction

Dragon fruit is a plant group of the *Cactaceae* family with the genus *Hylocereus* and also known as pitaya fruit is round, juicy, and nutritious (Jamilah, Shu, Kharidaah, Dzulkifly, & Noranizan, 2011; Idris et al., 2013). Swastika, Yuliani and Saputra (2012) explained that dragon fruit plants in Indonesia consist of several species, such as *Hylocereus undus, Hylocereus polyrhizus, Hylocereus megalanthus* and *Hylocereus costaricensis*. An important benefit of dragon fruit for health is to facilitate the digestion process because it has lots of fiber, reduces body fat levels, prevents cancer because it contains antioxidants, and stimulates tissue formation because it is rich in vitamins and minerals (Swastika et al., 2012; (Noor, Yufita, & Zulfalina, 2016).

In addition to the benefits above, dragon fruit has excellent sales prospects because prices in the market are high, stable and demand is high (Setiawan & Soelistyo, 2017; Sudarjat, Suminar, Qanit, & Mubarok, 2019; Paundrianagari, Setyowati, & Qonita,

2019). However, the superiority of the dragon fruit was not followed by good dragon fruit cultivation techniques by farmers. Therefore, many dragon fruit plants are attacked by diseases (Jumjunidang, D., & Yanda, 2016).

Dragon fruit diseases found in Indonesia include stem and fruit cancers, anthracnose, and soft rot. Stem and fruit cancers are caused by *Neocystalidium* sp., *Alternaria* sp., and *Pestaliopsis* sp. Anthracnose is caused by *Colletotrichum gloesporoides*. Stem and fruit soft rot caused by *Fusarium* sp., *Schlerotium* sp., *Alternaria* sp., and *Pseudomonas* sp. (Riska *et al,* 2016; Wibowo, Widiastuti, & Agustina, 2011; Swastika et al., 2012).

Recommendations for controlling microbial agents of dragon fruit disease currently using synthetic fungicidal compounds such as bordeux, propineb, copper hydroxide, benomil, mankozeb, and carbendazim (Riska et al., 2016; Jumjunidang et al., 2016; Widiastuti, Agustina, Wibowo, & Sumardiyono, 2011). However, pesticides have negative effects on other organisms that have an important role in the ecosystem and the environment (Syromyatnikov, Isuwa, Savinkova, Derevshchikova, & Popov, 2020; Maksymiv, 2015). So that these problems do not occur, it is recommended to use biocontrol agents and plant extracts to control plant pathogenic microbes (Abdel-Gaied, Mikhail, Abdel-Alim, Seif El-Nasr, & El-Khair, 2020).

One of the plants that can be recommended as an antimicrobial is zodia (*E. suaveolens*) because the results of isolation and identification of active compounds find essential oils that can be used as antimicrobials (Maryuni, 2008). Essential oils and other active compounds such as alkaloids, tannins, flavonoids, triterpenoids, saponins, glycosides, berberine, furoquinoline and evodiamine can inhibit growth and kill microbes (Fajri and Agustien, 2015); Rahmawati, Samsumaharto, & Iryanto, 2015; Handayani and Nurcahyanti, 2015). Furthermore, zodia plants (*E. suaveolens*) are shrubs that have a height of 50-200 cm that are easily cultivated through seeds or stem cuttings (Rahayu, Mairawita, & Putra, 2008) and this plant is a yard ornamental plant (Prayitno & Elan, 2018) making it easier to provide ingredients for antimicrobials. Research on antimicrobial compounds and the antimicrobial efficacy of zodia plants (Evodia suaveolens) has been conducted at several universities such as Maryuni (2008) from the Institut Pertanian Bogor, Handayani and Nurcahyanti (2015) from Universitas Negeri Semarang, Fajri and Agustien (2015) Universitas Andalas and Rahmawati, Samsumaharto, & Iryanto (2015) from Universitas Setia Budi Semarang.

Maryuni (2008) used steam distillation methods and Gas Chromatography-Mass Spectrometry (GCMS) to isolate antimicrobial compounds and with the disc diffusion method for antimicrobial testing of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Salmonella enteritidis*. Handayani and Nurcahyanti (2015) used the maceration method and water distillation to obtain antimicrobial compounds. Fajri and Agustien (2015) used the disc diffusion method for antimicrobial tests on *Escherichia coli* and *Staphylococcus aureus*. Rahmawati, Samsumaharto, & Iryanto (2015) used the maceration method to obtain antimicrobial compounds and delusional methods in test tubes.

Research on inhibiting the growth of microbes that cause soft rot in dragon fruit plants with plant extracts has not been done. Research Wibowo et al. (2011) and Riska et al. (2016) focused on collecting important diseases that attack dragon fruit plants in three centers of dragon fruit plants on the island of Java and in Indonesia. The use of zodia (*E. suaveolens*) leaf extract as an antimicrobial has not been studied. Although there is one result of research related to the antibacterial extract of zodia leaves against *P. aeruginosa* (Rahmawati, Samsumaharto, & Iryanto W, 2015). However, the study wanted to prove the comparison of zodia leaf extract with n-hexane, chloroform, and water fractions using the tube dilution method and recommended that the highest concentration of zodia leaf extract and be effective in inhibiting bacterial growth by 50%. However, this research is different from this

research. This research uses the disc-diffusion method and wells method with concentration that starts from 50% to 100%. Isolation and identification of zodia leaf antibacterial compounds was carried out (Maryuni, 2008; Handayani and Nurcahyanti, 2015; Rahmawati, Samsumaharto, & Iryanto, 2015). However, the active compounds contained in zodia leaf extracts have not been used to inhibit the growth of fungal pathogens such as *F. oxysporum*. Thus, the purpose of this study was to determine the antimicrobial activity of zodia (*E. suaveolens*) leaf extract on pathogenic agents of dragon fruit plants in vitro.

Materials and Methods

The research sample used fresh zodia (E. suaveolens) leaf and used all types of leaves both young and old, *P. aeruginosa* and *F. oxysporum*. The solvent used to extract zodia (E. suaveolens) leaves is 95% alcohol. Growth media for *P. aeruginosa* are Nutrient Agar (NA) and *F. oxysporum* is Potato Dextrose Agar (PDA). The tools used in research such as Petri dishes for the culture of bacteria and fungi. Oven for sterilizing utensils made of glass such as Petri dishes. Autoclave for sterilization of bacteria and fungi medium. Evaporator to obtain extracts of zodia leaf antimicrobial compounds. Calipers to measure the inhibition zone for bacterial and fungal growth. An ose needle to plant bacteria and fungi onto the plate medium. Laminar Air Flow (LAF) for sterile bacterial and fungal planting sites. Electric vortex to homogenize zodia leaf extract. Drill the cork to make a hole in the culture medium. Watman paper for disc paper material.

This research use a laboratory experimental method. The research design used Completely Randomized Design (CRD) with six concentrations of zodia (*E. suaveolens*) leaf extracts including 50%, 60%, 70%, 80%, 80%, 90%, and 100% and each consisted of four times repeated. The design of this study can be seen in Table 1. The independent variable of the study was zodia (*E. suaveolens*) leaf extract and the dependent variable was the zone of growth inhibition of *P. aeruginosa* and *F. oxysporum*.

Table 1. Research Design Design of antimicrobial activity of zodia (*E. suaveolens*) leaf extract

Treatment	Replicant			
	1	2	3	4
P1	P 1.1	P1. 2	P1. 3	P1. 4
P2	P 2.1	P2. 2	P2. 3	P2. 4
P3	P 3.1	P3. 2	P3. 3	P3. 4
P4	P 4.1	P4. 2	P4. 3	P4. 4
P5	P 5.1	P5. 2	P5. 3	P5. 4
P6	P 6.1	P6. 2	P6. 3	P6. 4
K (+)	K (+).1	K (+).2	K (+).3	K (+).4
K (-)	K (-).1	K (-).2	K (-).3	K (-).4

K (+) Fusarium oxysporum = synthetic antifungal; K (+) Pseudomonas aeruginosa = chlorampenicol; K (-) F. oxysporum and P. aeruginosa = aquadest

The research procedure is as follows. (1) sterilization of tools and materials. (2) Preparation of *P. aeruginosa and F. oxysporum* medium. (3) Regeneration of *P. aeruginosa* and *F. oxysporum*. (4) Making zodia (*E. suaveolens*) leaf extract through the maceration method with 95% alcohol solvent. (5) Making serial concentrations of zodia (*E. suaveolens*) leaf extracts. (6) Test the antimicrobial activity of zodia (E. suaveolens) leaf extracts against *P. aeruginosa* growth by disc-diffusion method and F. oxysporum by wells method.

The research instrument used was the observation sheet of *P. aeruginosa* and *F. oxysporum* growth diameter of inhibition zone. Inhibitory zones are indicated by clear zones and measured using calipers. The calipers are placed in the clear zone horizontally and vertically. Furthermore, the results of the measurement of the clear zone are averaged so that the expected inhibition zone is obtained. Diameter of inhibition zone data were analyzed using the One Way ANOVA test through SPSS software.

Results and Discussion

The average diameter of *P. aeruginosa* growth inhibition zones is presented in Table 1. The average diameter of *F. oxysporum* growth inhibition zones can be seen in Table 2. Research data on the antimicrobial activity of zodia (*E. suaveolens*) leaf extract is stated to be normally distributed (sig > 0, 05) and

homogeneous (sig > 0.05). The results of the One Way ANOVA test for diameter of inhibition zone of antimicrobial activity of zodia (*E. suaveolens*) leaf extracts against *P. aeruginosa* growth are presented in Table 3 and *F. oxysporum* can be seen in Table 4. The diameter of inhibition zone *P. aeruginosa* growth in several concentrations presented in Figure 1.

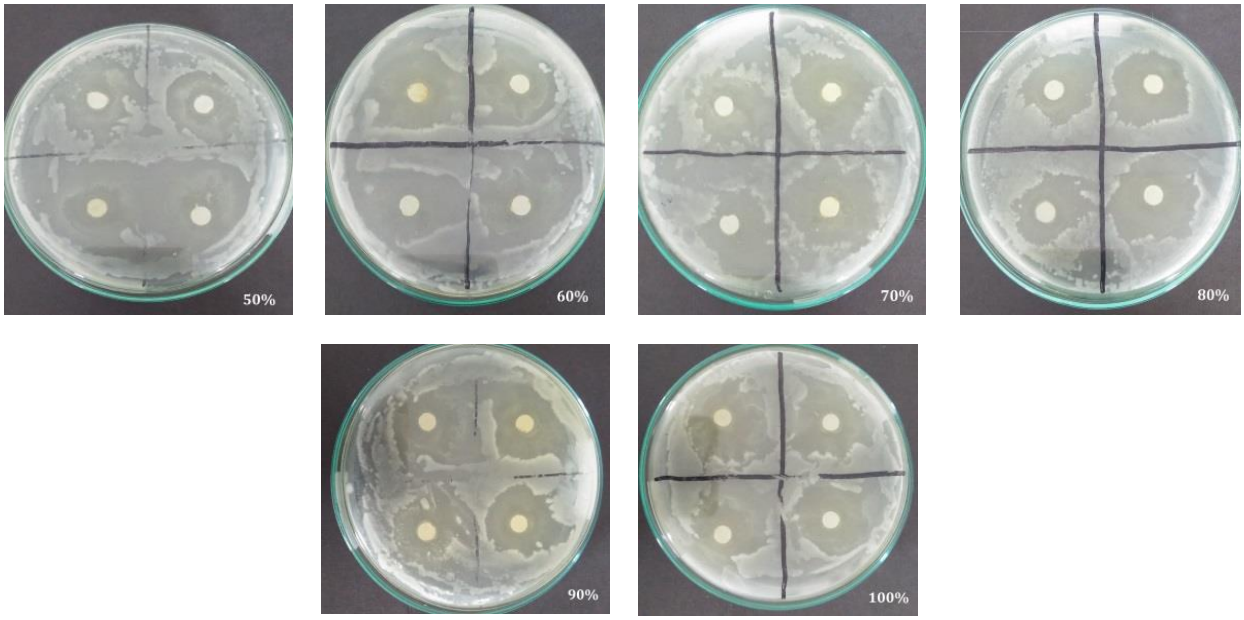

Figure 1. The Diameter of Inhibition Zone *P. aeruginosa* Growth in Several Concentrations of Zodia (E. suaveolens) Leaf Extract

Table 1. The Diameter of Inhibition Zone *P. aeruginosa* Growth

Replicant	Diameter of Inhibition Zone (Cm)					
	50%	60%	70%	80%	90%	100%
1	1,76	1,78	2,02	2,39	2,44	2,79
2	1,47	1,62	2,25	2,32	2,24	2,87
3	1,23	1,78	2,15	2,00	2,59	2,43
4	1,56	1,57	2,47	2,41	2,43	2,40
Average	1,51	1,69	2,22	2,28	2,43	2,62
Std. Deviation	0,22	0,11	0,19	0,19	0,14	0,24

Table 2. The Diameter of Inhibition Zone *F. oxysporum* growth

Replicant	Diameter of Inhibition Zone (Cm)					
	50%	60%	70%	80%	90%	100%
1	0,74	0,70	0,67	0,74	0,99	0,98
2	0,60	0,60	0,60	0,68	0,60	1,02
3	0,60	0,60	0,68	0,69	0,60	0,86
4	0,60	0,70	0,73	0,60	0,97	0,84
Average	0,64	0,65	0,67	0,68	0,79	0,93
Std. Deviation	0,07	0,06	0,05	0,06	0,22	0,09

Based on Table 1 and Table 2 shows that the higher the concentration of zodia (*E. suaveolens*) leaf extract, the greater the inhibitory power on the growth of *P. aeruginosa* and *F. oxysporum*. The high concentration of zodia (*E. suaveolens*) leaf extract contains many active compounds, while the concentration of zodia (*E. suaveolens*) leaf extract contains few active compounds. Therefore, 100% concentration of zodia (*E. suaveolens*) leaf extract able to provide the highest inhibition on the growth

of *P. aeruginosa* and *F. oxysporum*. The statement above corresponds to the statement Qomar, Budiyanto, Sukarsono, Wahyuni, & Husamah (2018) and Ali, Salih, & Daffalla (2020) that the higher the concentration of the extract, the more active compounds it contains so that it can produce high microbial growth inhibition. Asadi (2016) and Fikselova et al. (2014) added that antimicrobial activity was more commonly found at higher extract concentrations.

Table 3. Summary of One Way ANOVA Antimicrobial Activity of Zodia (*E. suaveolens*) Leaf Extract on *P. aeruginosa* Growth

Aspect	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25.53	7	3.65	128.32	.000
Within Groups	.68	24	.03		
Total	26.22	31			

Table 4. Summary of One Way ANOVA Antimicrobial Activity of Zodia (*E. suaveolens*) Leaf Extract on *F. oxysporum* Growth

Aspect	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.78	7	0.40	14.71	.000
Within Groups	.65	24	.03		
Total	3.43	31			

Tables 3 and 4 show that the significance value was 0,000 and smaller than 0.050 (P <0.050). This means that there is a significant inhibitory effect of the antimicrobial activity of zodia (*E. suaveolens*) leaf extract on the growth of P. aeruginosa and F. oxysporum.

Significant inhibitory effects of the antimicrobial activity of zodia extracts (*E. suaveolens*) are caused by the content of active compounds, such as essential oils, flavonoids, alkaloids, saponins, and tannins. The active compound contained in zodia (*E. suaveolens*) leaf extract acts as an antimicrobial. This statement is in line with the results of the study Rahmawati, Samsumaharto, & Iryanto (2015) states that zodia leaf extracts with n-hexane, chloroform, and water fractions contain active compounds including essential oils, flavonoids, alkaloids, saponins, and tannins that function as antibacterial agents to inhibit the growth of *P. aeruginosa*. Handayani and Nurcahyanti (2015) added that they had isolated essential oils that function as antimicrobial from zodia leaf using the water distillation method. Fajri and Agustien (2015) from their research have proven that the antimicrobial compounds from zodia leaf can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*.

Maryuni (2008) explained that active compounds of zodia (*E. suaveolens*) leaf extract such as essential oils and other active compounds can be designated as antimicrobials because they can inhibit the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Salmonella enteritidis*. Essential oils and other active compounds can damage bacterial cell walls and microbial cell membranes such as bacteria and fungi. Damage to cell walls and microbial cell membranes may cause the active compound extracts of zodia (*E. suaveolens*) leaf into the cell and affect the metabolic activities of bacterial cells. The metabolism of microbial cells becomes "error" resulting in death. Besides, damage to cell walls and microbial cell membrane makes molecules and compounds in the cytoplasm of cells out into the environment so that bacterial cells do not have the power to grow because all the molecules and enzymes needed for growth are lost.

Karta & Burhannuddin (2017); Pangalinan, Kojong, & Yamlean (2011); Soleman & Setiawan (2017); Sari & Nugraheni (2013); Triani, Rahmawati, & Turnip (2017); dan Yanti, Samingan, & Mudatsir (2016) states that antimicrobial active compounds contained in certain plant parts can increase cell membrane permeability and cell membrane damage. This causes interference with the metabolism of microbial cells so that they die. Konaté, Yomalan, Sytar, & Brestic (2015) and Alam, Forid, Roney, Aluwi, & Huq (2020) explains that flavonoids and tannins have important

antimicrobial activity because flavonoids can dissolve the constituents of cell walls while tannins can inactivate microbial cell enzymes and proteins.

Conclusion

The antimicrobial activity of zodia (*E. suaveolens*) leaf extract can significantly inhibit the growth of *P. aeruginosa and F. oxysporum* (P <0.05). The findings of this study recommend that zodia (*E. suaveolens*) leaf extract can be used as an antimicrobial. Furthermore, the antimicrobial of zodia (*E. suaveolens*) leaf extract can be applied in direct research in the field.

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